REMARKS

Applicant requests reconsideration of the application in view of the foregoing amendments and the discussion that follows. The status of the claims as of this amendment is as follows: Claims 44-46 are pending. Claims 1-43 were previously canceled. Applicant reserves the right to file divisional applications to the separately patentable subject matter of claims 7, 8, and 10-24, and Applicant generally reserves the right to file continuation applications to the subject matter of canceled claims 1-43. Claim 44 has been amended herein.

The Amendments

The specification was amended in several paragraphs to reinstate some of the language of the paragraphs prior to the previous amendment thereby addressing certain issues raised in the Office Action.

The paragraph on page 4, lines 20-30 was amended to delete the language "to a detectable product" in the second line of the paragraph.

The paragraph on page 9, lines 5-22, was amended to delete the language "the multiple substrates as" in the sixth sentence of the paragraph.

Claim 44 was amended to refer to a water-insoluble solid support. Support therefor is in the specification, for example, page 23, lines 18-19, and page 24, line 24. Claim 44 was also amended to clarify that it is the sensitizer that is capable in its excited stated of generating a reactive oxygen species. Support therefor is in the specification, for example, original claim 44. Claim 44 was also amended to delete the language referring to "substrate" as kindly suggested in the Office Action.

Objection to the Specification

Applicant submits that the foregoing amendments to the specification obviate the objections with regard to alleged introduction of new matter and to certain of the informalities raised in the Office Action. Asserted informalities not specifically addressed by amendment are discussed below.

The Office Action asserts that the mechanism by which release of the substrate with formation of a first binding site may be accompanied by unmasking of at least some

of a second binding site is unclear. The specification indicates that, in one embodiment of the invention, an oxidant cleavable linker may be used to attach to a support a substrate molecule having two binding sites wherein one of the binding sites is at least partially masked and is completely unmasked upon cleavage of the link and formation of the product. Masking is described in the specification as a situation whereby the functional group is unable to bind to its specific binding reagent. Such masking can arise simply by virtue of the substrate being bound to a surface. For instance, as set forth in the specification, the substrate may be bound within pores of the support or surface, i.e., an agarose gel, where the pores are too small to accommodate the specific binding reagent. Alternatively, numerous substrate molecules bound to a relatively smooth surface will be unavailable for binding to a specific binding reagent provided that the specific binding reagent is sufficiently bulky as, for example, when it is attached to latex particles. Thus, release of the substrate with formation of the first functional group or binding site may be accompanied by unmasking of at least some of the second functional group or binding site.

Particular examples of unmasking are also set forth in the specification. For example, the specification discusses a method for the selective protection or masking of biotin and analogues thereof at the ureido nitrogen using a singlet oxygen cleavable group. The method employs a copper catalyzed coupling reaction to couple the ureido nitrogen of biotin with a variety of unsaturated singlet oxygen sensitive compounds such as oxazole and anthracene halides, vinyl halides, and aryl halides. Deprotection or demasking of the biotin is accomplished in the presence of singlet oxygen, which cleaves off the masking group. The cleavable group may function as a protective mask to shield biotin in the presence of proteins such as avidin and streptavidin, which strongly bind to biotin. Alternatively, the cleavable group may function simultaneously as a linker to attach biotin to a molecule, support or surface and as a protective mask to shield the biotin in the presence of binding proteins. Singlet oxygen cleavage of the cleavable group simultaneously frees the biotin from the support or surface and unmasks the biotin, allowing the unmasked biotin to bind to an appropriate protein as desired.

The Office Action responded to Applicant's examples from the specification by asserting that Applicant's arguments were not persuasive. The Office Action contends that none of the examples are analogous to a mechanism involving both: (1) formation of a first binding site; and (2) unmasking of a second binding site.

Applicant respectfully traverses this contention. For instance, with regard to the example involving the selective protection or masking of biotin and analogues thereof at the ureido nitrogen using a singlet oxygen cleavable group, the specification indicates that release of the substrate with formation of the first functional group or binding site may be accompanied by unmasking of at least some of the second functional group or binding site. The specification indicates that singlet oxygen cleavage of the cleavable group simultaneously frees the biotin from the support or surface and unmasks the biotin, allowing the unmasked biotin to bind to an appropriate protein as desired. Accordingly, the free amino group on the biotin represents the formation of the first binding site and the unmasked biotin represents unmasking of the second binding site.

A similar situation is discussed in the specification as a fourth embodiment. A substrate attached to a support or surface via an oxidant cleavable linker reacts with an oxidant to produce a product having a chemically reactive group, usually an electrophilic group. This chemically reactive group is designed to react with a chemical-specific binding reagent, which can be a nucleophile such as an amine or sulfhydryl. The product therefore becomes covalently bound to the specific binding reagent. The product also contains a hapten or ligand that was originally present in the substrate or unmasked as a result of the oxidation or subsequent reaction. For example, oxidation of a substrate that contains one haptenic group linked to a support through an oxidizable linker can yield an active ester as the chemically reactive group. The specific binding reagent could then be an amine, which reacts with the ester. If the amine is attached to a label, reaction with the oxidation product not only releases the product from the support or polymer but also binds the product to the label. If the amine is not attached to a label, it can react with the product to produce a new group, which can serve as a ligand. A labeled receptor for the ligand can then be used in the subsequent detection step.

Rejection under 35 U.S.C. 112

Claims 44-46 were rejected under the second paragraph of the above code section as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Applicant believes that the amendments to claim 44 obviate this ground of rejection.

Rejection under 35 U.S.C. 102

Claims 1, 2 and 4-6 were rejected under paragraph (e) of the above code section as being anticipated by Bronstein, *et al.* (U.S. Patent No. 6,243,980) (Bronstein).

Bronstein discloses two distinct assays, namely, a heterogeneous assay and a homogeneous, each involving distinct reagents for each type of assay. As discussed by the patentee, a heterogeneous assay is described in the specification and illustrated in Fig. 3. At col. 5, line 42, *et seq.*, the patentee indicates that a peptide substrate is synthesized which contains the appropriate cleavage site for the target protease. This peptide is labeled with one member of a first ligand binding pair, such as biotin, on one end and a member of a second ligand binding pair, such as fluorescein, at the other end. This peptide is then incubated with the protease and a compound of interest to be screened for inhibitory activity, in a well or other solid phase coated with the second binding ligand of the first ligand binding pair, such as avidin or streptavidin. In this way, the biotin binds to the streptavidin and the fluorescein-labeled peptide becomes bound to the well unless the peptide was cleaved by the protease in which case only the biotin with a peptide fragment binds to the streptavidin in the well. After incubation, the wells are washed, incubated with the second binding member of the second binding ligand pair conjugated with an enzyme, which is an effective trigger for a 1,2-dioxetane such as alkaline phosphatase, washed, incubated with a 1,2-dioxetane substrate such as chlorine substituted phosphate dioxetane (CSPD) and the signal is measured. Higher signals are detected in the presence of an inhibitor. This is so because the inhibitor prevents the protease from cleaving the peptide and the fluorescein-peptide-biotin moiety bound to the streptavidin attached to the well remains intact. Therefore, the fluorescein is available for binding to the binding member for the fluorescein (second

binding member for the first binding member of the second ligand binding pair), which is conjugated to the alkaline phosphatase. Thus, the alkaline phosphatase reagent remains in the well after washing and is available to act on the CSPD substrate resulting in the release of a chemiluminescent species. If the test compound is not a protease inhibitor, the protease cleaves the peptide and there is no fluorescein-peptide-biotin moiety remaining in the well after washing.

Such an assay is unrelated to the presently claimed methods. This teaching by Bronstein is deficient in not disclosing at least the following limitations of claim 44: (1) a second specific binding pair member bound to a sensitizer where the sensitizer is capable in its excited state of generating a reactive oxygen species, wherein the proximity of first specific binding pair member with the second specific binding pair member is modulated by the presence of the analyte; (2) digoxigenin-linked biotin linked to the solid support through a reactive oxygen cleavable linker; (3) exciting the sensitizer where the excitation of the sensitizer causes the formation of reactive oxygen, which cleaves the cleavable linker and releases digoxigenin-linked biotin from the solid support; and (4) detecting the released digoxigenin-linked biotin, the amount thereof being related to the amount of analyte in the medium.

The Office Action asserts that the first specific binding pair member, referring to the alkaline phosphatase of Fig. 3, is bound to a support, identifying the adamantyl moiety as the support. Without acquiescing in this assertion, the adamantyl moiety is not a water insoluble solid support.

The Office Action identifies the second specific binding pair member as the $OPO_3^{=}$ in Fig. 3 bound to a sensitizer referring to a 1,2-dioxetane moiety precursor capable in its excited state of generating a reactive oxygen species, referring to photooxygenation of a dioxetane. This contention in the Office Action is not persuasive for a number of reasons. First, the disclosure in the reference concerning photooxygenation relates to the preparation of the CSPD reagent wherein oxygen is added to the double bond of the precursor to form the 1,2-dioxetane moiety. This preparation process has nothing to do with the claimed sensitizer capable in its excited state of generating a reactive oxygen species where the sensitizer is bound to a second member of a specific binding pair. As is readily seen, the CSPD is a formed reagent that

is used in the protease inhibitor assay. It is not a sensitizer reagent as presently claimed and does not generate a reactive oxygen species in the Bronstein assay, especially a reactive oxygen species that cleaves an oxygen-cleavable linker. Cleavages that occur in the heterogeneous assay of Bronstein are cleavage of the synthetic peptide reagent and cleavage of the 1,2-dioxetane. Cleavage of the synthetic peptide reagent results from the protease that is present during the initial part of the disclosed assay. Cleavage of the 1,2-dioxetane results from the action of the alkaline phosphatase on the $OPO_3^{=}$ of the CSPD reagent. Neither of these cleavages involves a reactive oxygen species generated by a sensitizer reagent or the cleavage of an oxygen-cleavable linker.

As mentioned above, Bronstein also does not disclose or suggest a method wherein excitation of a sensitizer causes the formation of reactive oxygen, which cleaves the cleavable linker and releases digoxigenin-linked biotin from the solid support. The Office Action asserts that the disclosure in the reference at col. 7, lines 10-12, discloses labels for either end of the peptide and include biotin and digoxigenin.

Applicant submits that the patentee is merely disclosing alternative labels that may be attached to the peptide. Even where biotin is employed as the first member of the first ligand binding pair linked to a fluorescein-labeled peptide substrate as disclosed in Bronstein at col. 5, lines 43-48, this reagent and its use in the protease inhibitor assay of the reference does not satisfy the claim language of claim 44. The claim recites exciting the sensitizer, which causes the formation of reactive oxygen that cleaves the cleavable linker and releases digoxigenin-linked biotin from the solid support and detecting the released digoxigenin-linked biotin. In the assay of the reference, cleavage, when it occurs, releases a peptide fragment with fluorescein attached but the biotin moiety is left bound to the streptavidin attached to the well. Accordingly, even if for the sake of argument digoxigenin were used in place of fluorescein (a proposition that is unsupported by any teaching in the reference), cleavage would release a peptide fragment with only digoxigenin attached and not biotin. Accordingly, a digoxigenin-linked biotin is not released from a solid support by a cleavage reaction and, furthermore, a digoxigenin-linked biotin is not detected.

Bronstein also discloses a synthetic peptide for a homogeneous assay (Fig. 14B). This peptide is derivatized at both ends of the peptide molecule. Fluorescein as

an energy accepting fluorescent moiety is at one end of the peptide and a 1,2-dioxetane moiety precursor (Fig. 14A) is at the other end. This reagent may be photooxygenated *in situ* to form the 1,2-dioxetane (Fig. 14B). The 1,2-dioxetane reagent is then employed in a homogeneous assay for the protease inhibitor. See, for example, the discussion at col. 7, lines 38-63, of Bronstein. The peptide is sufficiently short (no more than about 10 amino acid residues) such that the 1,2-dioxetane moiety is in close physical association with the fluorescent label. Upon triggering of the dioxetane, which can be effected by addition of an enzyme, or pH alteration, or application of heat or other triggers, the dioxetane decomposes, emitting energy that excites the fluorescent moiety, which then fluoresces if no cleavage of the peptide has occurred (a positive test for protease inhibition). If cleavage of the peptide has occurred, the dioxetane and fluorescent moieties are no longer in close physical relationship, and light is emitted by the chemiluminescent dioxetane. The wavelength of the fluorescent emitter is characteristically shifted markedly from that of the dioxetane, allowing easy discrimination in a homogenous assay.

The homogeneous assay of Bronstein is unrelated to the presently claimed methods. This teaching by Bronstein is deficient in not disclosing at least the following limitations of claim 44: (1) a second specific binding pair member bound to a sensitizer where the sensitizer is capable in its excited state of generating a reactive oxygen species, wherein the proximity of the first specific binding pair member with the second specific binding pair member is modulated by the presence of the analyte; (2) digoxigenin-linked biotin linked to the solid support through a reactive oxygen cleavable linker; (3) exciting the sensitizer where the excitation of the sensitizer causes the formation of reactive oxygen, which cleaves the cleavable linker and releases digoxigenin-linked biotin from the solid support; and (4) detecting the released digoxigenin-linked biotin, the amount thereof being related to the amount of analyte in the medium. The homogeneous assay disclosed by Bronstein is completely devoid of any teaching of the above limitations.

The Office Action has attempted to piece together portions of the above distinct assays of Bronstein in an effort to produce the presently claimed methods. As discussed above, even the pieced together portions are deficient in not teaching the

elements of the present claims. Furthermore, the Office is required to consider all that a reference discloses; piecemeal reconstruction of the prior art is not allowed. It is not permissible to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciation of what such reference fairly suggests to one of ordinary skill in the art. *In re Wesslan*, 147 USPQ 391, 827 O.G. 348 (1966).

Conclusion

Applicant has demonstrated that claims 44-46 satisfy the requirements of 35 U.S.C. 112 and 102. Furthermore, the specification is free of informalities as discussed above. Allowance of the above-identified patent application, it is submitted, is in order.

Respectfully submitted,

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